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Mechanism of Chenodeoxycholic Acid in Regulating Fatty Acid Metabolism and Maintaining Lipid Homeost

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Mechanism of Chenodeoxycholic Acid in Regulating Fatty Acid Metabolism and Maintaining Lipid Homeostasis

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A running head

CDCA regulates fatty acid metabolism and lipid homeostasis.

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28

29 CRediT authorship contribution statement

30 Lishuang Li, project design, data collection, and manuscript drafting; Xing Ren
31 and Xinyu Gao, Data collection, analysis, and manuscript contributions; Yujia
32 Zhao and Yuman Ma, Support in design, data collection, and method formulation;
33 Junxiao Gong and Shuo Yang, Analysis of vascular endothelial cells and writing
34 contributions; Yanan Sun and Yi Wang (Corresponding author), Overall project
35 supervision, guidance, data analysis, and manuscript writing.

36

37 Conflict of Interest Statement

38 The authors declare that the research was conducted in the absence of any commercial
39 or financial relationships that could be construed as a potential conflict
40 of interest.

41

42 Data availability

43 Data will be made available on request.

Abstract

Objective: To investigate the regulatory role and mechanisms of chenodeoxycholic acid (CDCA) on lipid metabolism, and to evaluate its therapeutic potential in lipid metabolism-related diseases such as acne, seborrheic alopecia, and prostate cancer.

Methods: Lipid-overproduction models were established using RM-1 (mouse prostate cancer) and primary sebaceous gland cells, stimulated by linoleic acid (LA) and dihydrotestosterone (DHT), respectively. CDCA's mechanisms were explored using transcriptomics, proteomics, and fatty acid-targeted metabolomics. In vivo validation was conducted via intradermal injection of CDCA into the sebaceous gland area of golden hamsters to assess its effect on sebaceous lipid metabolism.

Results: CDCA significantly reduced intracellular lipid accumulation in both murine prostate cancer cells (RM-1) and primary sebaceous gland cells, downregulating key lipogenic enzymes (SREBF1, FASN, FADS2), and suppressed androgen receptor (AR) expression. Mitochondrial membrane potential was restored in CDCA-treated cells. Multi-omics analyses revealed that CDCA modulated fatty acid biosynthesis and degradation, energy metabolism, mitochondrial function, and peroxisome proliferator-activated receptor gamma (PPAR- γ) signaling. Lipidomic profiling demonstrated a shift from saturated to unsaturated fatty acid dominance after CDCA treatment. In vivo, CDCA decreased sebaceous lipid accumulation and downregulated PPAR- γ expression in golden hamsters.

66 **Conclusion:** CDCA exerts multifaceted regulatory effects on lipid metabolism,
67 hormone signaling, and mitochondrial dynamics, maintaining sebaceous gland h
68 omeostasis and offering potential therapeutic value for disorders involving dysre
69 gulated lipid metabolism.

70 **Keywords**

71 Chenodeoxycholic acid (CDCA); Fatty acid metabolism; Lipid homeostasis

72 **Abbreviations**

Abbreviation	Full Name
ACADVL	Acyl-CoA dehydrogenase very long chain
ACC	Acetyl-CoA Carboxylase
ALDH2	Aldehyde dehydrogenase 2 family member
AR	Androgen receptor
CA	Cholic acid
CBR4	Carbonyl reductase 4
CDCA	chenodeoxycholic acid
DCA	Deoxycholic acid
DDA	Data-dependent acquisition
DHT	Dihydrotestosterone
DIA	Data-independent acquisition
ECGS	Epithelial cell growth supplement
FADS1-3	Fatty Acid Desaturases 1-3
FASN	Fatty Acid Synthase
HDL-C	High-density lipoprotein cholesterol
HE	Hematoxylin-eosin
HSD17B4	Hydroxysteroid 17-beta dehydrogenase 4
JC-10	Mitochondrial membrane potential probe
KRT7	keratin 7

LA Linoleic acid

36 NAFLD Non-alcoholic fatty liver disease

PCSK2 Proprotein convertase subtilisin/kexin type 2

13 PPAR- γ Peroxisome Proliferator-Activated Receptor Gamma

PSA Penicillin/streptomycin/amphotericin B

RM-1 Murine prostate cancer cells

32 SCD1 Stearoyl-CoA desaturase 1

SREBF1 Sterol Regulatory Element-Binding Transcription Factor 1

TC Total cholesterol

TG Triglycerides

UDCA Ursodeoxycholic acid

1. Introduction

Lipid homeostasis refers to the state in which organisms maintain the balance of lipid levels, encompassing multiple aspects such as fatty acid metabolism. As fundamental units of lipid metabolism, fatty acids play pivotal roles in critical physiological processes including energy supply, cellular membrane structure, and signal transduction[1-3]. However, dysregulation of lipid metabolism is closely associated with the pathogenesis of various diseases, particularly in cancer and dermatological disorders. For instance, in prostate cancer, aberrant lipid metabolism not only contributes to tumorigenesis but also correlates with malignant progression and therapeutic resistance[4-6]. In dermatology, disrupted lipid metabolism is implicated in acne and seborrheic alopecia. Excessive lipid accumulation drives acne pathogenesis, while abnormal lipid metabolism may alter the follicular microenvironment in seborrheic alopecia[7, 8]. Thus, elucidating the regulatory mechanisms of lipid homeostasis is essential for understanding disease mechanisms and developing novel therapeutic strategies.

Bear bile, a traditional Chinese medicine, has been widely utilized for its heat-clearing, detoxifying, liver-soothing, and vision-improving properties. Ancient texts such as the *Xiao'er Weisheng Zongwei Lun Fang (Compendium of Pediatric Health and Refined Prescriptions)* documented the use of "Xiongdan Shexiang Wan" (Bear Bile and Musk Pill) to treat pediatric malnutrition syndromes, stating: "For treating pediatric malnutrition syndromes, abdominal distension, pica, and persistent fever" [9]. The term "malnutrition syndromes" in moder

2 n contexts may correlate with metabolic disorders, including malnutrition and d
igestive dysfunction, both linked to lipid metabolism. Studies indicate that bear
3 bile powder improves lipid profiles by reducing total cholesterol (TC) and trig
lycerides (TG) while elevating high-density lipoprotein cholesterol (HDL-C) lev
els[10-12]. Furthermore, clinical research demonstrates that bear bile-containing
formulations and bear bile powder itself exhibit efficacy in managing lipid met
abolism disorders such as hyperlipidemia and non-alcoholic fatty liver disease
(NAFLD) [13, 14]. These findings highlight bear bile's potential in modulating
fatty acid metabolism and maintaining lipid homeostasis.

Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), the pri
mary bioactive components of bear bile powder[15-17], have shown therapeutic
promise in metabolic disorders. Both bile acids improve hepatic function, regu
late cholesterol metabolism, and mitigate inflammation, with established clinical
applications in NAFLD and primary biliary cholangitis[18-20].

However, the precise molecular mechanisms by which CDCA regulates fatt
y acid metabolism—particularly in lipid-related pathologies such as cancer and
sebaceous gland disorders—remain incompletely understood. Notably, the effects
of CDCA on sebaceous lipid metabolism have not been systematically explore
d. It is hypothesized that CDCA can restore lipid homeostasis by regulating ke
y fatty acid metabolic pathways in both tumor and sebaceous gland systems. T
his study is, to the best of current knowledge, the first to integrate transcripto
mic, proteomic, and fatty acid-targeted metabolomic analyses to investigate the

118 regulatory effects of CDCA on lipid metabolism in both RM-1 prostate cancer
119 cells and primary sebaceous gland cells. In addition, a golden hamster model
120 was employed to validate CDCA's influence on sebaceous lipid production in
121 vivo, thereby bridging in vitro mechanistic insights with preclinical evidence.

122 This study systematically investigates the effects of CDCA on lipid metabo
123 lism in both cancerous and sebaceous systems. Murine prostate cancer cells (R
124 M-1) and primary sebaceous gland cells were used to establish lipid-overproduc
125 tion models, induced by linoleic acid (LA) and dihydrotestosterone (DHT), resp
126 ectively. Through transcriptomics, proteomics, and fatty acid-targeted metabolom
127 ics, the regulatory mechanisms of CDCA were explored. Additionally, an *in viv*
128 o golden hamster model was employed to verify CDCA's effect on sebaceous
129 lipid metabolism via intradermal injection.

130 This study expands current understanding of bile acid pharmacology by re
131 vealing novel mechanisms through which CDCA modulates fatty acid metabolis
132 m in both cancer and skin systems. These findings extend the therapeutic lands
133 cape of CDCA beyond hepatic applications, offering mechanistic and translation
134 al insights into lipid-driven dermatological and oncological disorders.

135 2. Materials and Methods

136 2.1 Cell Culture of RM-1 and primary sebaceous gland cells

137 RM-1 cells (CL-0198, Procell Life Science & Technology, China) and pri
138 mary sebaceous gland cells (CTCC-280-HUM, Meisen Cell Technology, China)
139 were cultured in a humidified incubator at 37°C with 5% CO₂. Humidity was

maintained by placing 3000 mL of sterile distilled water in the incubator to prevent medium evaporation. RM-1 cells were cultured in DMEM medium (SH30022.01, HyClone Laboratories, USA) supplemented with 10% FBS and 1% penicillin/streptomycin/amphotericin B (PSA). primary sebaceous gland cells were maintained in specialized epithelial cell medium (CTCC-009-613, Meisen Cell Technology, China), composed of basal medium, 10% FBS, 1% epithelial cell growth supplement (ECGS), and 1% penicillin/streptomycin. Cells within passages 4-8 were used for experiments. Routine subculturing was performed when cells reached 80% confluency.

STR profiling confirmed that the sebaceous gland cells were of human origin, consistent with the supplier's documentation. In addition, immunostaining for keratin 7 (KRT7)—a cytoskeletal protein expressed in epithelial lineages including sebaceous cells—was used to further support their identity (Supplementary Material 3). All STR authentication results are provided in Supplementary Materials 1 – 3.

2.2 CCK-8 Assay

RM-1 cells in the logarithmic growth phase were trypsinized, centrifuged, and resuspended in culture medium. Cell suspensions (8,000 cells/well) were seeded into 96-well plates and incubated at 37°C with 5% CO₂ until adherent. Cholic acid (CA, 81-25-4, Macklin Biochemical Co., Ltd., China), deoxycholic acid (DCA, 83-44-3, Macklin Biochemical Co., Ltd., China), UDCA (128-13-2, Macklin Biochemical Co., Ltd., China), and CDCA(474-25-9, Macklin Biochemi

cal Co., Ltd., China) were diluted in serum-free medium to concentrations of 0–500 µmol/L. After 24 h of treatment, 10 µL of CCK-8 solution (CK04, Dojindo Laboratories, Japan) and 90 µL of serum-free medium were added to each well. Plates were gently shaken to ensure uniform distribution and incubated for 30 min at 37°C. Absorbance was measured at 450 nm using a microplate reader (Synergy H1, BioTek, USA). Cell proliferation rates were calculated as:

Cell viability (%) = [(Experimental group – Blank control) / (Negative control – Blank control)] × 100.

2.3 Nile Red Staining for Lipid Detection

RM-1 and primary sebaceous gland cells in the logarithmic phase were seeded into 96-well plates (8,000 cells/well) and allowed to adhere overnight. Groups included: normal control, LA-treated (0.1 mmol/L for RM-1; 0.0125 mmol/L for primary sebaceous gland cells), and LA combined with CA/DCA/UDCA/CDCA. After 24 h of treatment, cells were exposed to 20 µg/mL dihydrotestosterone (DHT, D413176, Aladdin Biochemical Technology Co., Ltd., China) for 48 h. Medium was discarded, and cells were washed three times with PBS (5 min each). Cells were fixed with 4% paraformaldehyde (50 µL/well, 20 min), washed with PBS, stained with Nile Red (7385-67-3, Solarbio Life Sciences, Beijing, China) (50 µL/well, 20 min, dark), and washed again. Triton X-100 (0.5%, 50 µL/well, 5 min) and DAPI (5 min) were sequentially applied. Fluorescence was visualized using an inverted microscope (CKX41, Olympus, Japan).

184

2.4 Mitochondrial Membrane Potential Assay

Cells were treated for 24 h, washed with PBS, and incubated with 1 mL mitochondrial membrane potential probe (JC-10) staining working solution (CA1310, Solarbio Life Sciences, Beijing, China) at 37°C for 20 min. JC-10 staining buffer (1×) was prepared by diluting 5× stock with distilled water (1:4) and stored on ice. After incubation, cells were washed twice with 1× buffer and observed under a fluorescence microscope.

192

2.5 qPCR Analysis of Lipid Metabolism Genes in RM-1

Total RNA was extracted using TRIzol reagent (15596018, Invitrogen, USA), reverse-transcribed with ReverTra Ace qPCR RT Master Mix (FSQ-201, TOYOBO, Japan), and quantified. qPCR was performed using SYBR Green Pre mix (PK-201, TOYOBO, Japan) in 20 µL reactions (95°C for 30 sec, 40 cycles). Gapdh served as the reference gene. Relative mRNA levels were calculated via the $2^{-\Delta\Delta C_t}$ method. The PCR primer (Shanghai Sangon Biotech Co., Ltd., China) sequences used in this study are listed in Supplementary Material 4.

201

2.6 Western Blot Analysis

After 24 hours of treatment, cells were harvested for protein extraction. Protein lysates were normalized based on concentration, mixed with loading buffer, and denatured at 100 °C for 10 minutes. Approximately 20–40 µg of protein

(in a volume of 5–15 μ L) per lane was separated by SDS-PAGE at 200 V. After the bromophenol blue front reached the gel bottom, proteins were transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk and incubated overnight at 4 °C with primary antibodies against fatty acid synthase (FASN), sterol regulatory element-binding transcription factor 1 (SREBF1), FADS2, and Histone 3 (all diluted 1:1000). Notably, the anti-FASN (A19050), anti-FADS2 (A24354), and anti-Histone 3 (A4835) antibodies were obtained from China Abclonal Biotechnology Co., Ltd, while the anti-SREBF1 antibody (14088-1-AP) was purchased from Proteintech (USA). The next day, membranes were washed thrice with TBST (10 minutes each) and incubated with HRP-conjugated secondary antibodies (1:1000) at room temperature for 1 hour, followed by further TBST washes. Signal detection was performed using ECL reagents and visualized on a ChemiDoc imaging system (BIO-RAD, USA).

219

2.7 Multi-Omics Profiling

2.7.1 RNA-Seq

Cell samples from RM-1 were flash-frozen in liquid nitrogen, ground, and lysed in 1 mL of pre-chilled Trizol. After incubation at room temperature for 5 minutes, samples were centrifuged, and the supernatant was transferred to a new tube. Next, 200 μ L of pre-chilled chloroform was added, mixed vigorously, and allowed to stand for 5 minutes at room temperature. Following another centrifugation, the aqueous phase was collected, mixed with an equal volume of

7 228 f pre-chilled isopropanol, and incubated for 10 minutes. After centrifugation, th
7 229 e RNA pellet was washed with 1 mL of 75% ethanol, briefly air-dried (3–5 m
7 230 inutes), and resuspended in 20–50 μ L of 0.1% DEPC-treated water. The total
231 RNA was then used for mRNA enrichment, fragmentation, cDNA synthesis, ad
232 apter ligation, and subsequent sequencing on the Illumina NovaSeq Xplus platf
233 orm. Data analysis—including read alignment, expression quantification, differen
234 tial expression analysis, and KEGG enrichment using the Python SciPy package
235 —was performed. Please refer to Supplementary Material 5 for details.

236

237 2.7.2 Fatty Acid Metabolomics

238 primary sebaceous gland cells samples (300 μ L per sample, diluted 2 $\times$) w
239 ere stored at -80°C . A mixed standard solution of 50 common fatty acids was
240 prepared at 2000 $\mu\text{g/mL}$ and further diluted using a 50% acetonitrile–isopropa
241 nol solution. Isotopically labeled internal standards were also prepared. Fatty ac
242 ids were extracted using acetonitrile and isopropanol to ensure efficient recover
243 y. Quantification was performed using UHPLC-MS/MS (AB SCIEX ExionLC™
29 244 AD system coupled with a QTRAP® 6500+ mass spectrometer) in multiple re
245 action monitoring mode. Standard curves generated from known concentrations
246 were used to determine sample concentrations, with statistical analyses performe
247 d using Microsoft Excel and appropriate statistical software.

248

249 2.7.3 Deep data-independent acquisition (DIA) Proteomics

Proteins from primary sebaceous gland cells samples were extracted and analyzed using an Orbitrap high-resolution mass spectrometer in a DIA workflow. This method enables simultaneous collection of fragment data from all precursor ions, providing enhanced coverage and reproducibility compared to data-dependent acquisition (DDA). Quantitative analysis was conducted using DIA-NN software with retention time calibration via internal peptide standards. Differentially expressed proteins were defined as those with expression changes greater than 1.2-fold or less than 0.83-fold and a P-value < 0.05. Functional annotation and enrichment analyses were carried out using the COG, GO, and KEGG databases, and protein-protein interaction networks were constructed to elucidate biological functions.

261

2.8 Golden Hamster Model

Three SPF-grade golden hamsters (average weight ~120 g, 8 weeks old) were procured from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. and housed in the Animal Experimental Facility of the Institute of Basic Theory, China Academy of Chinese Medical Sciences (License No. SCXX (Beijing) 2021-0006). The animals were maintained under controlled environmental conditions (temperature 20–24 °C, humidity 40–60%, 12-hour light/dark cycle, with adequate ventilation, standardized feed, and ad libitum water). The hair on the sebaceous gland regions of both sides of the abdomen was removed prior to experimentation. The experimental group received subcutaneous injection

s of a CDCA injection solution in the right-side sebaceous gland region, prepared per 2 mL as follows: 2 mg CDCA, 18 mg benzyl alcohol, 2.84 mg disodium hydrogen phosphate, 8.76 mg sodium chloride, 2.86 mg sodium hydroxide, and injection water, with pH adjusted to 8.3 as needed using hydrochloric acid and sodium hydroxide. The control group was administered an equivalent volume of blank control solution. Injections were given daily for six consecutive days, and data were recorded at various time points.

279

2.9 Dermatoscopic Data Acquisition

Following induction of anesthesia with isoflurane, the dorsal hair of the golden hamsters was removed using depilatory cream and thoroughly washed with physiological saline. Once the skin was dry, a medical electronic dermatoscope (CH-DSIS-2000, Chuanghong Medical Technology, China) equipped with a 20× lens was placed in close contact with the skin. The focus was adjusted until a clear image of the sebaceous gland region was obtained, and parameters such as gland size and pigment deposition were recorded.

288

2.10 Oil Red O Staining

Frozen sections (10 µm thick) of the sebaceous gland region were prepared and fixed in 10% formalin for 30 minutes. Sections were then rinsed twice with distilled water (3 minutes each), treated with 60% isopropanol for 20–30 seconds, and stained with an improved Oil Red O staining kit (G1261, Solarbi

53 294 o Life Sciences, Beijing, China) for 10–15 minutes. After brief differentiation
295 with 60% isopropanol (5–10 seconds) and a distilled water rinse (1 minute), M
296ayer's hematoxylin was applied for 1–2 minutes. The sections were then washe
297d, air-dried, and mounted with glycerol gelatin. Lipid droplets appeared in shad
298es of orange-red, while nuclei were stained blue.

299 2.11 Hematoxylin-eosin (HE) Staining

300 Paraffin sections (4 μm thick) were deparaffinized and rehydrated prior to
301 staining with an HE staining kit (G1120, Solarbio Life Sciences, Beijing, Chin
302a). Sections were stained with hematoxylin for 2 minutes, rinsed under running
303 water for 10 minutes to achieve “blueing,” briefly differentiated with an acidi
304c solution for 10 seconds, and subsequently stained with eosin for 20 seconds.
305 After thorough rinsing, the slides were dehydrated, cleared in xylene, and mo
306unted with neutral gum for optical microscopy image acquisition.

308 2.12 Immunofluorescence Staining

309 Frozen sections (10 μm thick) from the sebaceous gland region were equil
46 310 ibrated to room temperature and washed three times with PBS (3 minutes per
41 311 wash). After blocking with 5% goat serum at room temperature for 1 hour to
312 prevent nonspecific binding, sections were incubated overnight at 4 °C with pri
313 mary antibodies against Ki67 (dilution 1:200) and Cytokeratin 5 (K5, dilution
314 1:200) (27309-1-AP and 66727-1-Ig, respectively, from Proteintech, China). The
3 315 following day, sections were washed three times with PBS, incubated with the

corresponding secondary antibody (1:1000) at room temperature for 1 hour, and finally mounted with DAPI-containing fluorescence mounting medium. Images were captured using a fluorescence microscope. Fluorescence imaging was performed using a touchscreen microscope (RVL2-K2, BICO, USA) with high-resolution objectives (4x–100x oil) and multi-wavelength LED excitation. Standard fluorophore filter sets (e.g., DAPI, FITC, TRITC, Cy5) were used, and images were captured with a 5 MP CMOS camera (2448×2048 pixels). Acquisition and processing were controlled via Revolve software, enabling automated exposure, focus, and filter selection. Image analysis was performed on three independent biological replicates. For each replicate, four images were captured under consistent exposure settings. Two images were averaged to generate one data point, resulting in six statistical values per group ($n = 6$). This design ensures both data representativeness and statistical reliability.

2.13 Statistical Analysis

All images were analyzed using ImageJ software (Version 1.8.0, NIH) with standardized protocols. Immunofluorescence intensity was quantified by threshold-based methods (auto-threshold algorithm combined with negative control calibration) to calculate integrated density. Oil Red O staining was processed via RGB color thresholding (Hue 0–30, Saturation 50–255) to determine lipid droplet-positive area percentage (%Area). Prior to analysis, uniform background subtraction and region of interest (ROI) delineation were performed.

28 338 Data were statistically evaluated using GraphPad Prism 8.0. Initial tests for
339 normality (Shapiro–Wilk) and homogeneity of variances (Levene’s test) were p
340 erformed; if assumptions were violated, non-parametric tests or variance adjust
21 341 ments were applied. Differences between groups were analyzed using one-way
342 ANOVA, followed by Bonferroni or Tukey post hoc tests for multiple comparis
15 343 ons. A *P*-value of <0.05 was considered statistically significant.
344

345 3. Result

62 346 3.1 Regulatory Effects of CDCA on Lipid Metabolism in RM-1 Cells

347 3.1.1 Effects of Different Bile Acids on RM-1 Cell Viability and LA-Induce 348 d Lipid accumulation: CDCA's Potent Inhibitory Effect and Concentration 349 Advantage

51 350 To systematically evaluate the effects of bile acids on RM-1 cell viability,
351 the concentration-gradient responses of CA, DCA, UDCA, and CDCA were a
352 nalyzed using CCK-8 assays (Figure 1A–D). The IC₁₀, IC₁₅, and IC₂₀ concentra
353 tions were selected from SPSS-predicted inhibition ranges (IC₁₀–IC₂₅) to enable
354 pharmacological comparisons under equivalent inhibition conditions while maint
355 aining >80% cell viability for physiologically relevant lipid metabolism studies.

356 As shown in Figure 1A–D, all bile acids exhibited clear concentration-depende
357 nt inhibitory effects, with CDCA demonstrating the strongest potency, as eviden
358 ced by its significantly lower IC₁₀ concentration (0.05 μmol/L) compared to ot
33 359 her compounds (*P*<0.05, one-way ANOVA). Notably, statistically significant diff

erences ($P<0.05$) were observed between high-concentration groups (IC₂₀) and 0 $\mu\text{mol/L}$ controls.

To assess the combined effects of bile acids and linoleic acid (LA, 0.1 mmol/L), their suppression of LA-induced lipid accumulation at IC₁₀/IC₁₅/IC₂₀ concentrations was further assessed (Figure 1E–K). Nile red staining revealed that CDCA and UDCA exhibited markedly stronger lipid-suppressive effects than CA and DCA. Strikingly, CDCA exhibited a 52-fold greater potency than UDCA, achieving comparable lipid suppression at a significantly lower IC₁₀ concentration (0.05 $\mu\text{mol/L}$ vs. 2.6 $\mu\text{mol/L}$). Given its superior concentration-efficacy profile and robust lipid-modulatory capacity, CDCA was prioritized for subsequent mechanistic investigations.

Figure 1

3.1.2 Multiple Regulatory Effects of CDCA on Lipid Metabolism, Mitochondrial Membrane Potential, and androgen receptor (AR) Expression in RM-1 Cells

As illustrated in Figure 2, CDCA significantly inhibited lipid accumulation induced by linoleic acid (LA, 0.1 mmol/L) in RM-1 cells (Figures 2A–B). Quantitative analysis of Nile red fluorescence intensity revealed a marked reduction in lipid accumulation in the CDCA-treated group compared to the LA group.

Magnified images further demonstrated that CDCA alleviated localized intracellular lipid droplet aggregation (Figure 2C).

Given the close relationship between lipid synthesis and mitochondrial energy metabolism, mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using JC-10 staining (Figures 2D–E). LA stimulation significantly increased $\Delta\Psi_m$, indicated by enhanced red fluorescence and diminished green fluorescence, suggesting elevated energy metabolism. In contrast, CDCA treatment significantly reduced the elevated $\Delta\Psi_m$ induced by LA, bringing it closer to baseline levels observed in untreated cells. Quantitative analysis of the JC-10 red-to-green fluorescence ratio confirmed that CDCA-treated cells exhibited significantly lower $\Delta\Psi_m$ than LA-stimulated cells.

Although CDCA simultaneously suppressed lipid accumulation and modulated $\Delta\Psi_m$, the causal relationship between these effects remains to be elucidated.

LA-induced lipid synthesis may elevate energy demands, thereby augmenting $\Delta\Psi_m$. However, CDCA's inhibitory effects extend beyond lipid synthesis suppression and may involve indirect mechanisms, such as modulating fatty acid metabolism or mitochondrial function, to influence $\Delta\Psi_m$. Thus, the interplay between reduced lipid synthesis and $\Delta\Psi_m$ attenuation likely involves a complex regulatory network requiring further investigation.

Furthermore, considering the close interplay between lipid metabolism and hormonal signaling in prostate cancer, AR overexpression in RM-1 cells was induced using DHT. After 48 hours of DHT stimulation, both AR expression and

d lipid accumulation were significantly elevated (Figures 2G–H). Additionally, CCK-8 proliferation assays demonstrated that DHT not only enhanced lipid accumulation but also increased cellular viability (Figure 2F).

Importantly, co-treatment with CDCA and DHT substantially reduced DHT-induced AR upregulation (Figures 2I–K). These findings suggest that CDCA may regulate lipid synthesis and metabolism by modulating androgen signaling through AR suppression. This mechanistic insight underscores CDCA's potential therapeutic value in lipid metabolism-related disorders, such as acne. Acne pathogenesis is closely linked to increased androgen levels and altered lipid metabolism. Elevated androgens, particularly DHT, stimulate sebaceous glands to produce excessive sebum, leading to clogged pores and promoting the growth of *Cutibacterium acnes*, a bacterium implicated in acne inflammation. Additionally, lipid metabolic dysfunction impairs the skin barrier and fosters an inflammatory environment conducive to acne development.

Moreover, under 2-DHT treatment, lipid accumulation increased, while CDCA was able to suppress lipid accumulation concurrently with AR inhibition (Figures 2L–M), further elucidating the positive correlation between AR and lipid metabolism.

Figure 2

3.1.3 CDCA Modulates Lipid Metabolism in RM-1 Cells via Regulation of Fatty Acid and Energy Metabolic Pathways

RNA sequencing analysis revealed that co-treatment with CDCA and LA significantly altered gene expression profiles in RM-1 cells (Figure 3A). Compared to the LA-treated group, the CDCA+LA group exhibited 72 upregulated and 57 downregulated genes (Figure 3C). KEGG pathway enrichment analysis indicated that these differentially expressed genes (DEGs) were predominantly associated with lipid and fatty acid metabolism-related pathways (Figure 3D). Notably, the enrichment of the fatty acid metabolism pathway underscores CDCA's pivotal role in regulating fatty acid synthesis, degradation, and metabolic homeostasis. Additionally, the enrichment of TGF- β and FoxO signaling pathways suggests that CDCA may influence lipid metabolism through indirect mechanisms.

The involvement of the GnRH signaling pathway further implies that CDCA could modulate fatty acid metabolism via endocrine regulatory mechanisms. Additionally, pathways related to energy metabolism, including ATP-dependent chromatin remodeling, were enriched, suggesting a potential link between CDCA treatment and alterations in cellular energy homeostasis. Furthermore, the enrichment of pathways like amino sugar and nucleotide sugar metabolism suggests potential interactions between carbohydrate and lipid metabolism in the regulation of fatty acids.

Further KEGG enrichment analysis focused on lipid metabolism-related pathways (Figure 3E). The results demonstrated significant enrichment in pathways including fatty acid metabolism, fatty acid degradation, biosynthesis of unsaturated fatty acids, cholesterol metabolism, glycerophospholipid metabolism, and bi

le acid biosynthesis. Energy metabolism-related pathways, such as the AMPK signaling pathway and glycolysis/gluconeogenesis, were also closely associated with lipid metabolism. Sankey diagram analysis identified key genes involved in these differential pathways, including aldehyde dehydrogenase 2 family member (ALDH2), acyl-CoA dehydrogenase very long chain (ACADVL), carbonyl reductase 4 (CBR4), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), and Protein convertase subtilisin/kexin type 2 (PCSK2) (Figure 3F). Specifically, ACADVL and HSD17B4, involved in fatty acid metabolism and degradation pathways, suggest that CDCA may influence lipid metabolism by regulating the fatty acid β -oxidation pathway. CBR4, associated with the biosynthesis of unsaturated fatty acids, further emphasizes CDCA's role in regulating fatty acid composition. Additionally, genes related to cholesterol metabolism and bile acid biosynthesis, such as ALDH2, indicate that CDCA may indirectly participate in the regulation of lipid homeostasis by affecting cholesterol and bile acid metabolism.

Building upon RNA-Seq analyses and previous experimental findings, The regulatory effects of CDCA on lipid and fatty acid metabolism were further investigated. Key genes associated with lipid synthesis include SREBF1 and Acetyl-CoA Carboxylase (ACC), while genes pivotal in fatty acid metabolism encompass FASN and Fatty Acid Desaturases 1-3 (FADS1-3). We assessed the mRNA expression levels of these critical factors (Figure 3G-L) and observed that CDCA significantly downregulated their expression in a concentration-dependent

manner. Western blot analysis further confirmed the downregulation of lipid metabolism-related proteins, including FASN, SREBF1, and FADS2, upon CDCA treatment (Figure 3M). Collectively, these findings suggest that CDCA modulates lipid metabolism by downregulating the expression of genes and proteins involved in lipid and fatty acid metabolism, highlighting its potential application in regulating lipid metabolic processes.

Figure 3

3.2 In Vivo and In Vitro Validation of CDCA Regulation on Sebaceous Gland and Lipid Metabolism

3.2.1 Regulation of Sebaceous Gland Activity and Lipid accumulation by CDCA in Golden Hamsters

To elucidate the mechanisms underlying CDCA's effects on lipid metabolism, this study utilized golden hamsters—an animal model characterized by a well-developed sebaceous gland system. Upon intradermal injection of CDCA into the sebaceous gland region, dermoscopic examination revealed a slight, non-significant reduction in the area of this region, accompanied by mild local inflammation and decreased melanin deposition (Figure 4A). Oil Red O staining indicated that CDCA significantly inhibited lipid accumulation in the sebaceous gland area, indicating its regulatory role in lipid metabolism (Figures 4B–C). Further histological analysis through hematoxylin and eosin (H&E) staining showed no significant morphological changes in sebaceous gland tissue structure, sug

gesting that CDCA does not cause structural damage to the glands; however, a noticeable reduction in gland size was observed (Figures 4D–E). Immunofluorescence staining revealed a significant decrease in Ki67 expression in peripheral sebaceous gland cells, indicating reduced cellular activity (Figure 4G). Additionally, the expression levels of AR and peroxisome proliferator-activated receptor gamma (PPAR- γ), both closely associated with lipid metabolism, were diminished following CDCA treatment. This finding aligns with observations in RM-1 cells, where CDCA not only regulates lipid metabolism but also affects AR expression. These results suggest that CDCA may modulate sebaceous gland homeostasis by influencing peripheral sebaceous gland cell activity, as well as androgen accumulation and the expression of lipid-related regulatory proteins such as PPAR- γ .

In summary, the study demonstrates that CDCA regulates sebaceous gland homeostasis by reducing lipid accumulation and cellular activity, potentially through the modulation of AR and PPAR- γ . Importantly, this effect occurs without disrupting the overall glandular structure, providing a theoretical basis for the application of CDCA in treating sebaceous gland-related disorders.

Figure 4

3.2.2 Regulation of Fatty Acid Composition and Lipid accumulation by CDCA in primary sebaceous gland cells

512 Building upon previous *in vivo* findings demonstrating that CDCA inhibits
513 lipid accumulation and AR expression in the sebaceous glands of golden ham
514 sters, its effects on sebaceous gland cells were further investigated using a pri
515 mary sebaceous gland cell model.

516 Lipidomic analysis (Figure 5A) revealed that CDCA treatment significantly
517 altered the fatty acid composition in primary sebaceous gland cells, including i
16 518 ncreased levels of various unsaturated fatty acids (such as linoleic acid and ara
519 chidonic acid) and polyunsaturated fatty acids, while decreasing certain saturate
520 d fatty acids (like palmitic acid). These changes suggest that CDCA optimizes
16 521 the fatty acid profile by enhancing anti-inflammatory fatty acids (e.g., linoleic
522 acid, which promotes skin barrier repair) and reducing pro-inflammatory saturat
523 ed fatty acids.

524 Further Deep DIA proteomic analysis (Figure 5B) unveiled the regulatory
525 mechanisms of CDCA. KEGG pathway enrichment analysis indicated that CDC
526 A modulates pathways related to fatty acid metabolism and degradation, includi
527 ng β -oxidation and biosynthesis of unsaturated fatty acids. Additionally, CDCA
528 significantly impacts mitochondrial function-related pathways (such as ATP synt
529 hesis and mitochondrial metabolism), implying its influence on lipid metabolism
530 through mitochondrial energy regulation.

531 A CCK-8 assay identified a non-toxic concentration of CDCA for primary
532 sebaceous gland cells (25 $\mu\text{mol/L}$) (Figure 5C). Nile Red staining (Figures 5E a
533 nd G) further confirmed that CDCA inhibits lipid accumulation in primary seba
534 cous gland cells. Under linoleic acid (0.0125 mmol/L) stimulation, CDCA signif
535 icantly suppressed lipid accumulation, demonstrating its direct regulatory effect
536 on sebocyte lipid metabolism.

CDCA reduced mitochondrial membrane potential in primary sebaceous gland cells, mirroring the effects observed in RM-1 cells. Moreover, considering the association between lipid accumulation and androgens, CDCA decreased AR expression and lipid accumulation induced by 2-DHT (Figures 5H–K), aligning with effects observed in RM-1 cells.

In summary, CDCA regulates sebaceous lipid metabolism through multiple mechanisms, including altering fatty acid composition, modulating metabolic pathways, affecting mitochondrial function, and concurrently regulating AR expression. These findings underscore CDCA's pivotal role in lipid metabolism regulation and its potential therapeutic value in treating lipid metabolism-related disorders.

Figure 5

4. Discussion

Lipid metabolism plays a central role in maintaining essential physiological processes such as cellular function, energy balance, and signal transduction. Dysregulation of lipid metabolism not only affects normal physiological functions but is also closely associated with the onset and progression of various diseases[11,21,22]. Recent studies have demonstrated that CDCA, a primary active component of bear bile, exhibits significant potential in regulating lipid metabolic disorders[20]. This study further investigates the role of CDCA in lipid metabolism regulation. We systematically examined the effects of CDCA on lipid me

559 tabolism using various cell models, including RM-1, primary sebaceous gland ce
560 lls, and *in vivo* experiments in the sebaceous gland area of golden hamsters. T
561 he results indicate that CDCA significantly inhibits lipid accumulation across th
562 ese different cell and animal models, primarily by modulating fatty acid metab
563 olism, energy metabolism, and mitochondrial oxidative phosphorylation.

564 Initially, CDCA's regulatory effects on lipids were validated using two cell
565 lines. In RM-1 cells, CDCA significantly inhibited LA-induced lipid accumulat
566 ion, exhibiting noticeable effects even at lower concentrations (IC₁₀, 0.05 μmol/
567 L), outperforming other bile acids such as UDCA. RNA-Seq analysis revealed
568 that CDCA's impact on RM-1 lipid metabolism is primarily focused on the reg
569 ulation of fatty acid metabolism and energy metabolism. PCR and Western blot
3 570 results indicate that CDCA significantly downregulates the mRNA and protein
571 expression levels of key genes such as SREBF1, ACC, FASN, FADS1-3, and
26 572 stearoyl-CoA desaturase 1 (SCD1). Inhibition of ACC and FASN directly affe
26 573 cts de novo fatty acid synthesis, while downregulation of FADS and SCD1 ma
574 y reduce the production of unsaturated fatty acids, thereby influencing the com
575 position of intracellular fatty acids. In primary sebaceous gland cells, lipidomics
576 analysis further confirmed CDCA's regulatory effects on fatty acid composition
577 [23-25]. CDCA treatment significantly upregulated the content of various unsatu
578 rated fatty acids (e.g., LA, arachidonic acid) while inhibiting the expression of
579 certain saturated fatty acids (e.g., palmitic acid). This regulation not only optim

izes fatty acid composition but may also improve skin condition by increasing the proportion of anti-inflammatory fatty acids (e.g., LA).

Our study not only reveals CDCA's regulation of fatty acid metabolism but also systematically explores its effects on energy metabolism and mitochondrial function for the first time. In RM-1 cells, a significant decrease in mitochondrial membrane potential was observed following CDCA treatment, suggesting that CDCA may influence the cellular energy metabolic state by regulating mitochondrial function. RNA-Seq analysis further supports this view, showing significant changes in energy metabolism-related pathways such as ATP-dependent chromatin remodeling after CDCA treatment. This aligns with recent research published in Nano Letters, which demonstrated that CDCA-loaded nanoparticles can reduce adipocyte size by inducing mitochondrial functions, including promoting β -oxidation[26]. Proteomic results from primary sebaceous gland cells also confirm this conclusion, indicating that CDCA not only affects fatty acid metabolism but also significantly regulates processes related to mitochondrial function and oxidative phosphorylation. This consistent effect across different cell types suggests that CDCA may influence energy metabolism and mitochondrial function through a universal mechanism, thereby regulating fatty acid metabolism and balance. Additionally, studies by Liu J et al. have shown that CDCA may promote lipid peroxidation by activating the ROS/p38 MAPK/DGAT1 axis and can directly bind to mitochondria, inducing mitochondrial changes in AML cells, including swelling and cristae reduction[27]. Our findings complement this disc

602 overy, collectively revealing CDCA's multifaceted role in regulating energy met
603 abolism and mitochondrial function.

604 Through RNA-Seq and proteomic analyses, several signaling pathways pote
605 ntially involved in CDCA's mechanism of action were identified. The AMPK s
606 ignaling pathway is likely a key mechanism by which CDCA regulates lipid m
607 etabolism. Additionally, changes in TGF- β and FoxO signaling pathways sugges
608 t that CDCA may influence lipid metabolism by regulating cell differentiation a
609 nd metabolism. Notably, CDCA's impact on the GnRH signaling pathway was
610 also observed, which may explain its regulatory effects on hormone-related lipi
611 d metabolism. In the experimental validation section, RM-1 cells were stimulate
612 d with DHT to induce AR overexpression, and CDCA was found to reduce A
613 R expression as well. This indicates that CDCA can regulate hormone expressi
614 on while modulating lipids, further confirming the close relationship between a
615 bnormal lipid accumulation and hormone levels.

616 Comprehensive results indicate that CDCA exhibits bidirectional regulation
617 of fatty acid metabolism. On one hand, it inhibits fatty acid synthesis by down
618 regulating genes such as SREBF1 and ACC. On the other hand, Deep DIA an
619 alysis suggests that CDCA may promote fatty acid degradation by activating β -
620 oxidation phosphorylation-related pathways. This bidirectional regulatory mechan
621 ism enables CDCA to more precisely balance intracellular fatty acid levels; mo
622 reover, it can optimize fatty acid composition, providing a theoretical basis for
623 its potential application in diseases related to lipid metabolic disorders.

Based on the research findings and existing literature, CDCA emerges as a versatile regulator of lipid metabolism with promising therapeutic applications. In skin-related lipid metabolism disorders, CDCA may hold potential value in the treatment of acne and seborrheic alopecia. Our observations in primary sebaceous gland cells indicate that CDCA suppresses lipid accumulation and optimizes fatty acid composition, providing direct evidence for its application in these conditions. By reducing sebum accumulation and increasing the proportion of anti-inflammatory fatty acids, CDCA may help alleviate acne symptoms[7,28]. In the context of seborrheic alopecia, CDCA's regulation of sebaceous gland activity could assist in controlling excessive oil accumulation. In diseases associated with lipid metabolism abnormalities, such as prostate cancer, CDCA's potential role warrants attention. Our observations in RM-1 prostate cancer cells demonstrate CDCA's inhibitory effect on lipid metabolism and its regulation of AR expression, offering new insights into its application in prostate cancer treatment. CDCA may inhibit tumor cell growth and metastasis by modulating lipid metabolism and hormone signaling pathways[29-31].

In summary, CDCA's consistent effects across various cell models, its comprehensive regulation of lipid metabolism, and its impact on energy metabolism and mitochondrial function lay the foundation for its potential application in the treatment of multiple diseases. Future research should further explore CDCA's mechanisms of action, optimize administration methods, and conduct preclinical

645 al and clinical studies to fully harness its potential in treating lipid metabolism
646 -related diseases.

647

648 5. Study Strengths and Limitations

649 Strengths of the Study

650 ● Multi-level validation system: This study integrated transcriptomics, proteom
651 ics, and fatty acid-targeted metabolomics to comprehensively elucidate the r
652 egulatory mechanisms of CDCA on lipid metabolism across multiple model
653 s, including RM-1 prostate cancer cells, primary sebaceous gland cells, and
654 *in vivo* hamster skin.

655 ● Bidirectional regulatory mechanism: CDCA was found to not only inhibit d
656 e novo fatty acid synthesis (via SREBF1, ACC, FASN) but also enhance f
657 atty acid degradation pathways (such as β -oxidation), suggesting a bidirecti
658 onal regulation of lipid metabolism.

12 659 ● Novel mitochondrial insights: The study is among the first to highlight the
660 mitochondrial regulatory role of CDCA in energy metabolism, supported b
661 y consistent findings in transcriptomics, proteomics, and JC-10 staining resu
662 lts.

663 ● Disease relevance and translational potential: Findings demonstrate CDCA's
664 applicability in two disease contexts—acne/seborrheic disorders and prostat
665 e cancer—thereby bridging dermatological and oncological therapeutic oppor
666 tunities.

Limitations of the Study

- Lack of receptor-level validation: Although CDCA's lipid regulatory effects were demonstrated, the study did not validate whether these effects are mediated through classical bile acid receptors such as FXR or TGR5.
- Limited mechanistic dissection: Key regulatory pathways (e.g., AMPK, MAPK) were predicted through omics but not directly confirmed via pharmacological inhibition or genetic manipulation.
- *In vivo* validation restricted to sebaceous model: While sebaceous regulation was validated *in vivo* using hamsters, the prostate cancer-related findings remain confined to *in vitro* data, limiting translational insights.
- No long-term toxicity or safety data: Although CDCA has known clinical applications, its long-term safety in topical or targeted dermatological use has not been assessed in this study.

6. Conclusion

CDCA demonstrates potential in modulating lipid metabolism by reducing lipid accumulation and reshaping fatty acid composition in RM-1 prostate cancer cells and sebaceous gland cells. It also influences mitochondrial function and energy metabolism, with in vivo validation confirming its regulatory effect on sebaceous gland homeostasis. These findings suggest CDCA may serve as a promising candidate for treating acne and seborrheic disorders, and offer mechanistic insight into its potential application in lipid-related cancers. Further studies are warranted to optimize its delivery and evaluate its safety and efficacy for clinical translation.

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Figure 1

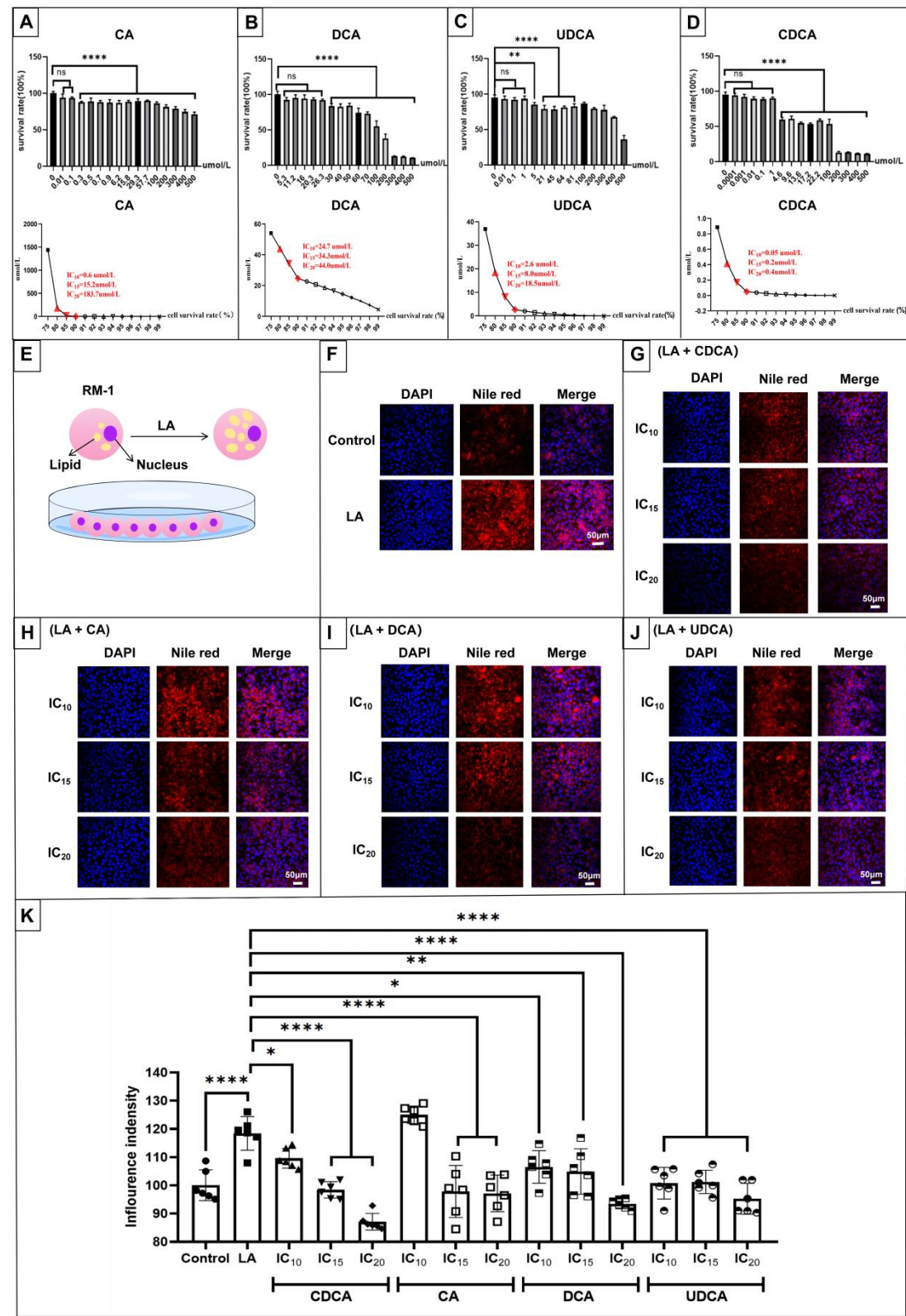


Figure 1. Effects of bile acids on RM-1 cell viability and LA-induced lipid accumulation.

A–D. CCK-8 assay results depicting the cytotoxic effects of CA (A), DCA (B), UDCA (C), and CDCA (D) on RM-1 cells across concentrations ranging from 0 to 500 $\mu\text{mol/L}$. Bar graphs represent cell viability percentages, while line graphs illustrate dose-response curves with calculated IC_{10} , IC_{15} , and IC_{20} values highlighted in red ($n = 5$); E. Schematic illustration of lipid accumulation in RM-1 cells stimulated with 0.1 mmol/L linoleic acid (LA) (A); F. Nile red staining of RM-1 cells after 24 h treatment with or without LA, indicating increased lipid accumulation upon LA exposure, nuclei were stained with DAPI (blue), and lipids with Nile red (red), scale bar = 50 μm ; G–J. Nile red staining of RM-1 cells co-treated with LA and each bile acid at IC_{10} , IC_{15} , and IC_{20} concentrations for 24 h: CDC A (G), CA (H), DCA (I), and UDCA (J), nuclei were stained with DAPI (blue), and lipids with Nile red (red), scale bar = 50 μm ; K. Quantification of Nile red fluorescence intensity ($n = 6$). Statistical analysis was performed using one-way ANOVA ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$).

Figure 2

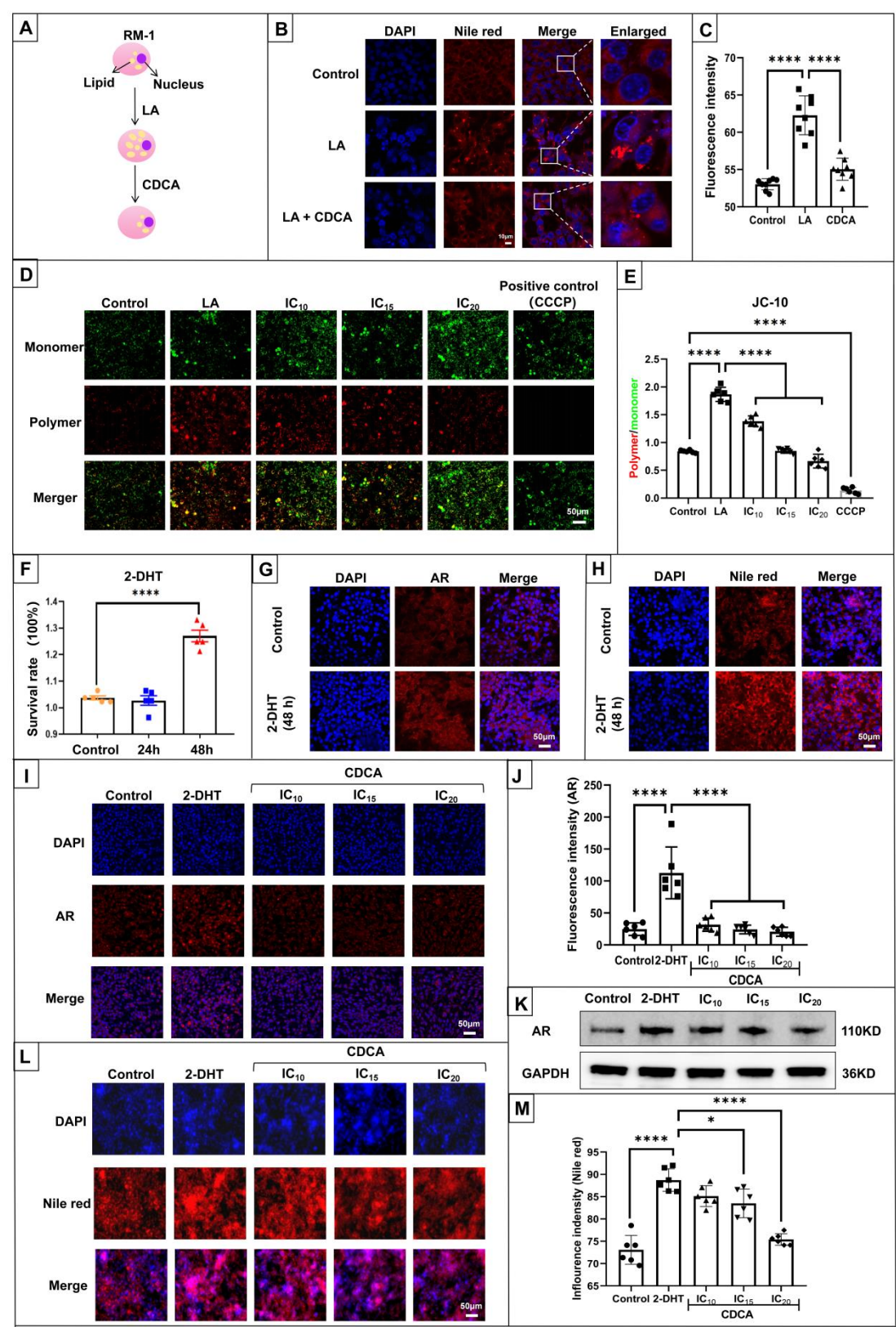
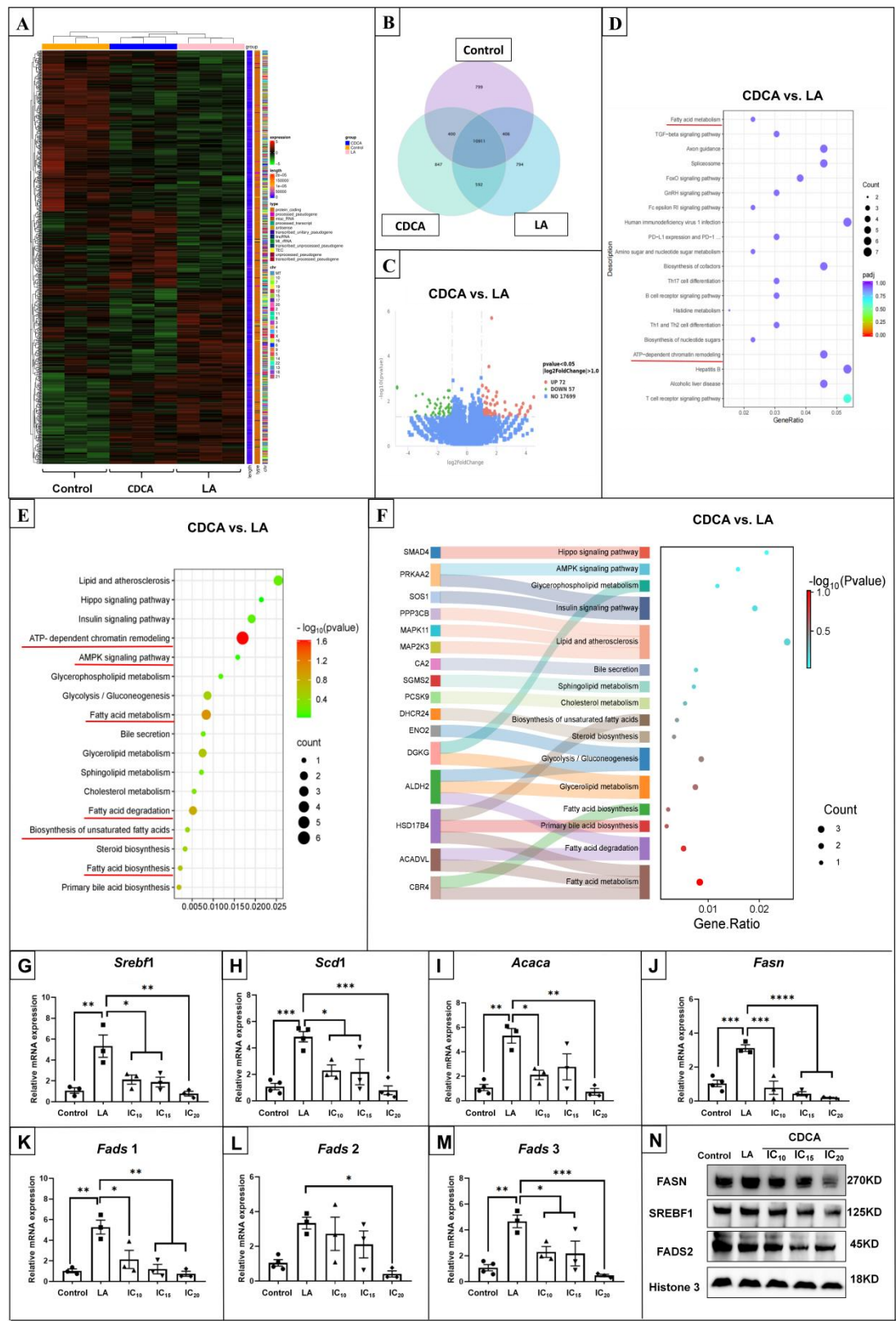


Figure 2. Multiple Regulatory Effects of CDCA on Lipid Metabolism, Mitochondrial Membrane Potential, and AR Expression in RM-1 Cells

A. Schematic model of lipid metabolic regulation in RM-1 cells: LA stimulation promotes lipid accumulation, while CDCA treatment reverses this effect; B. Nile Red staining of lipid droplets in RM-1 cells treated with LA or LA+CDCA (IC₂₀). Right panel shows magnified view of lipid droplet-rich regions (scale bar = 10 μ m); C. Quantitative analysis of Nile Red fluorescence intensity (n = 8); D. JC-10 staining illustrating mitochondrial membrane potential ($\Delta\Psi$ m) in RM-1 cells under various treatments: control, LA (0.1 mmol/L), CDCA (IC₂₀), and CCCP (positive control). Red fluorescence indicates JC-10 aggregates (intact $\Delta\Psi$ m), while green fluorescence represents monomers (depolarized $\Delta\Psi$ m). Scale bar = 50 μ m.; E. Quantitative analysis of JC-10 aggregate/monomer ratio (n = 6); F. Cell proliferation assessed by CCK-8 assay at 24 h and 48 h post-DHT stimulation (n = 6); G. Immunofluorescence staining of AR in DHT-treated RM-1 cells (48 h). Red: AR; blue: DAPI (scale bar = 50 μ m); H. Nile Red staining of lipid accumulation in DHT-stimulated RM-1 cells (scale bar = 50 μ m); I. AR immunofluorescence in cells co-treated with DHT and CDCA (IC₁₀-IC₂₀) (scale bar = 50 μ m); J. Quantitative analysis of AR fluorescence intensity (n = 6); K. Western blot analysis of AR protein expression in RM-1 cells co-treated with DHT and CDCA (IC₁₀-IC₂₀). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control, the original Western blot bands are provided in Supplementary Material 6; L. Nile Red staining of RM-1 cells co-treated with DHT and CDCA (IC₁₀-IC₂₀); M. Quantitative analysis of Nile Red fluorescence intensity (n = 6). Statistical analysis was

824 as performed using one-way ANOVA ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.$
825 0001).

Figure 3



826 **Figure 3. Multi-Level Mechanisms of CDCA-Regulated Lipid Metabolism in RM-1 Cel**

827 **ls**

828 A. Heatmap illustrating gene expression differences among the control, LA-treated, and CD

829 CA+LA-treated groups in RM-1 cells; B. Venn diagram showing the overlap of differential

830 ly expressed genes among the three experimental groups in RM-1 cells; C. Volcano plot d

831 epicting differentially expressed genes between the CDCA+LA and LA groups in RM-1 ce

832 lls (criteria: $P < 0.05$, $|\log_2\text{FoldChange}| > 1.0$); D. KEGG pathway enrichment analysis hig

833 hlighting significantly enriched metabolic and signaling pathways among the differentially e

834 xpressed genes in RM-1 cells; E. Bubble plot of KEGG enrichment for lipid metabolism-r

835 elated differential pathways; bubble size represents the number of genes associated with ea

836 ch pathway, and color indicates the significance of enrichment (expressed as $-\log_{10}(P\text{-valu$

837 e)) in RM-1 cells; F. Sankey diagram illustrating specific genes involved in the significantl

838 y enriched lipid metabolism-related pathways identified in panel E, and depicting the funct

839 ional distribution of these genes across different pathways in RM-1 cells; G–M. Quantitati

840 ve PCR analysis of relative mRNA expression levels of key genes involved in lipid synth

841 esis (*Srebf1*, *Acaca*, *Fasn*) and fatty acid metabolism (*Scd1*, *Fads* 1–3) in RM-1 cells acro

842 ss different treatment groups. Gapdh served as the internal control. Data are presented as

843 mean $\pm$ standard error ($n = 3$). N. Western blot analysis showing the expression levels of

844 lipid metabolism-related proteins (FASN, SREBF1, FADS2) in RM-1 cells, with Histone

845 3 serving as the internal control, the original Western blot bands are provided in Supplem

846 entary Material 6. Statistical significance was assessed using one-way ANOVA compared t

2

847 o the LA group.. Significance indicators: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P <$

848 0.0001.

Figure 4

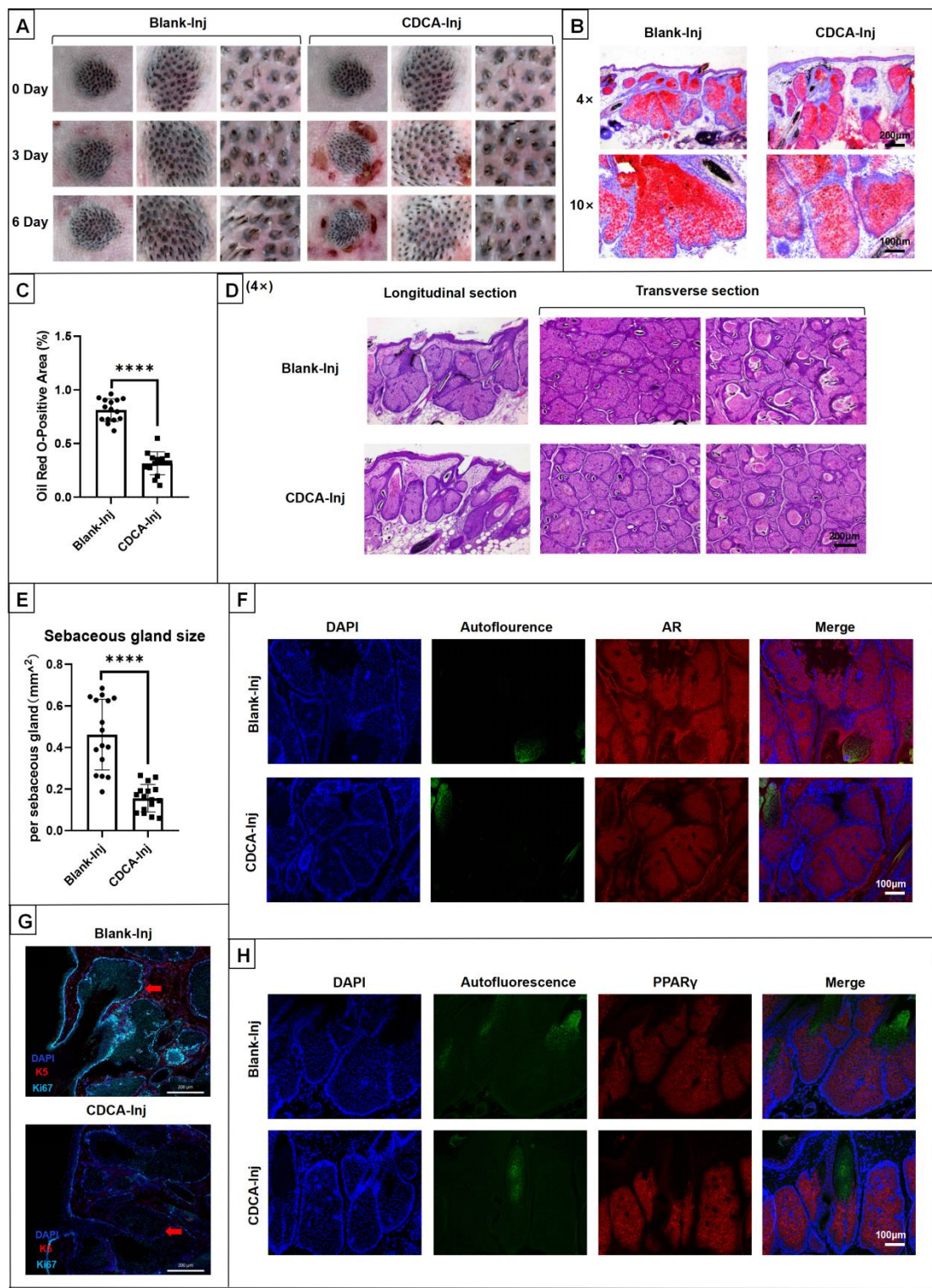


Figure 4. Effects of CDCA on Sebaceous Lipid accumulation and Cell Activity in Golden Hamsters

851 A. Dermoscopy images of the sebaceous gland region at days 0, 3, and 6 following CDC
852 A injection, presented with progressively magnified views from left to right; B. Oil Red O
853 staining of sebaceous gland tissue sections captured under 4× (scale bar = 200 μm) and 1
20 854 0× (scale bar = 100 μm) magnifications; C. Quantification of lipid droplet area percentage
855 based on Oil Red O staining (n = 15), statistical analysis performed using t-test; D. Hema
856 toxylin and eosin (H&E) staining of sebaceous gland tissue sections, including both transv
43 857 erse and longitudinal views, illustrating sebaceous gland morphology (scale bar = 200 μm);
858 E. Quantitative analysis of sebaceous gland size corresponding to the H&E-stained section
859 s in panel D (n = 15), statistical analysis performed using t-test; F–H. Immunofluorescenc
860 e staining of sebaceous gland tissues: nuclei are stained blue (DAPI), while target proteins
3 861 —AR and Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ)—are labeled in red.
862 Scale bar = 100 μm; G. Immunofluorescence staining of sebaceous gland sections: Ki67
863 (light blue) marks proliferating peripheral sebaceous gland cells (indicated by red arrows),
864 Keratin 5 (K5, red) labels sebaceous gland cells, and DAPI (deep blue) stains nuclei, scal
11 865 e bar = 200 μm. Statistical significance is indicated as follows: * $P < 0.05$; ** $P < 0.01$; **
866 * $P < 0.001$; **** $P < 0.0001$.

Figure 5

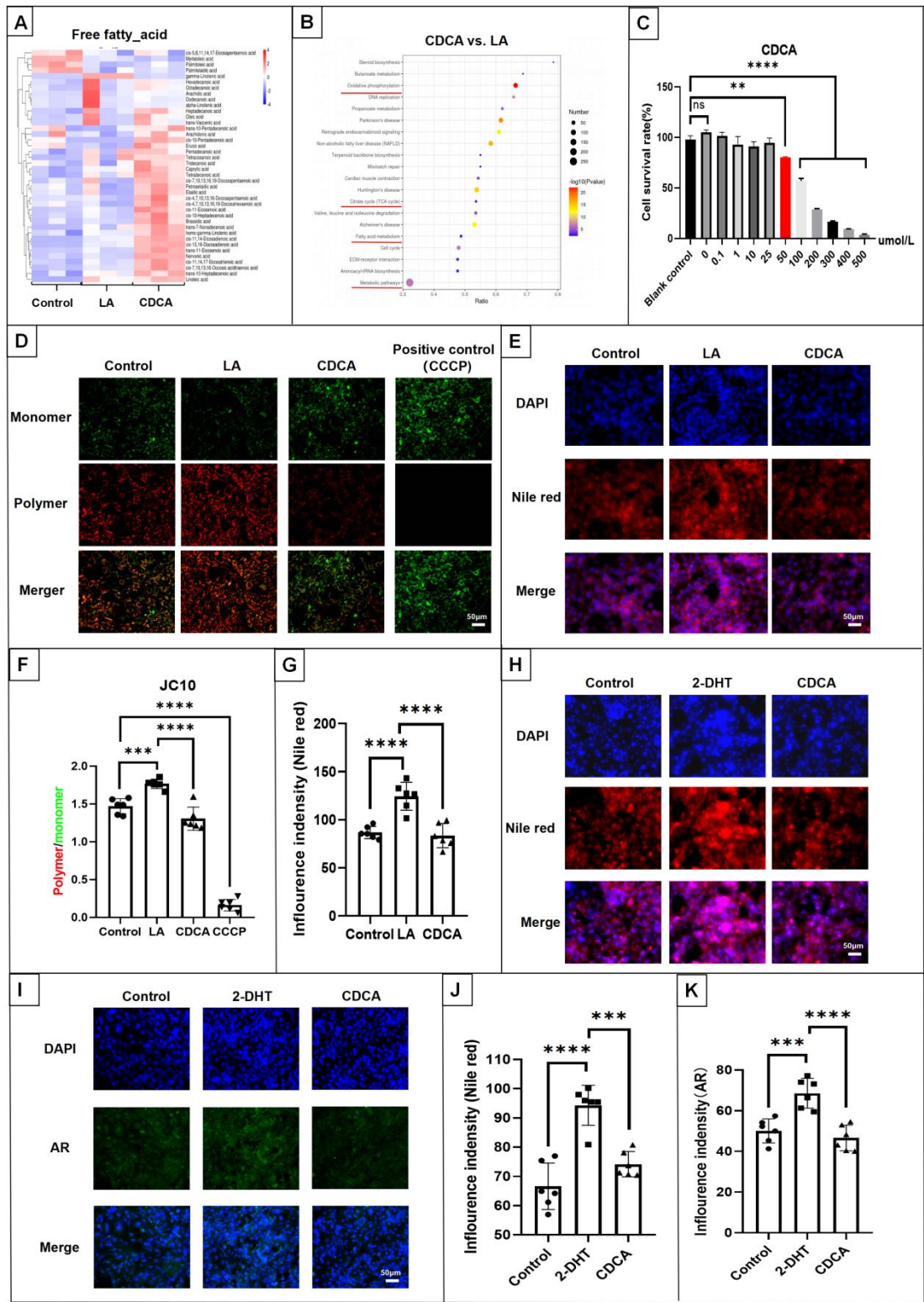


Figure 5. Effects of CDCA on Lipid Metabolism and Fatty Acid Composition in primary sebaceous gland cells Sebocytes

869 A. Heatmap displaying differential fatty acid profiles in primary sebaceous gland cells amo
870 ng control, LA-treated, and CDCA-treated groups; B. KEGG enrichment bubble plot of dif
871 ferentially expressed pathways from deep DIA proteomic analysis between CDCA- and LA
872 -treated groups; C. CCK-8 assay in primary sebaceous gland cells treated with CDCA (0–5
873 00 $\mu\text{mol/L}$) demonstrating cytotoxicity at 50 $\mu\text{mol/L}$. The non-cytotoxic 25 $\mu\text{mol/L}$ concentra
874 tion was selected for subsequent experiments; D. JC-10 mitochondrial membrane potential
875 staining in primary sebaceous gland cells following LA and CDCA treatment (scale bar: 50
876 μm); E. Nile Red fluorescence staining showing lipid droplets in primary sebaceous gland c
877 ells after 24-hour treatment with LA (0.0125 mmol/L) and CDCA (25 $\mu\text{mol/L}$) (scale bar: 5
878 0 μm); F. Quantification of mitochondrial membrane potential (red/green fluorescence ratio)
879 corresponding to panel D ($n = 6$); G. Nile Red fluorescence intensity quantification corre
880 sponding to panel E ($n = 6$); H. Nile Red staining (red) of primary sebaceous gland cells
6 881 co-treated with 2-DHT and CDCA, with DAPI nuclear counterstaining (blue) (scale bar: 5
882 0 μm , $n = 6$); I. AR immunofluorescence (green) in primary sebaceous gland cells co-treate
6 883 d with 2-DHT and CDCA, with DAPI nuclear staining (blue) (scale bar: 50 μm , $n = 6$).J.
884 Nile Red: Fluorescence Quantification Analysis (Corresponding to Figure H, $n=6$); K. AR:
4 885 Fluorescence Quantification Analysis (Corresponding to Figure I, $n=6$). Statistical signifi
886 cance was determined by one-way ANOVA versus control group: $*P < 0.05$; $**P < 0.01$; *
887 $**P < 0.001$; $****P < 0.0001$.